A SUBFAMILY OF RNA HELICASES WHICH ARE MODULATORS OF THE FIDELITY OF TRANSLATION TERMINATION AND USES THEREOF

DOMESTIC PRIORITY CLAIMED

The priority is claimed of U.S. Provisional Application No. 60/093,685, filed on July 22, 1998, which is hereby incorporated by reference herein in its entirety.

GOVERNMENT RIGHTS CLAUSE

The research leading to the present invention was supported, at least in part, by a grant from The National Institutes of Health (GM48631-01). Accordingly, the Government may have certain rights in the invention.

FIELD OF THE INVENTION

The present invention relates to a subfamily of RNA helicases, one of which is the MTT1 gene, which modulates the fidelity of translation termination. The present invention relates to a multiprotein surveillance complex comprising MTT1, human Upf1p, Upf2p, Upf3p, eucaryotic Release Factor 1 and eucaryotic Release Factor 3 which is involved in modulation of the efficiency of translation termination and degradation of aberrant mRNA. Identification of this complex provides an *in vitro* assay system for identifying agents that: affect the functional activity of mRNAs by altering frameshift frequency; permit monitoring of a termination event; promote degradation of aberrant transcripts; provide modulators (inhibitors/stimulators) of peptidyl transferase activity during initiation, elongation, termination and mRNA degradation of translation. Such agents which may be antagonists or agonists, are useful for screening, and diagnostic purposes, and as therapeutics for diseases or conditions which are a result of, or cause, premature translation.

BACKGROUND OF THE INVENTION

The translational apparatus is responsible for synthesizing cellular proteins. This machinery must be able to determine the precise sites on the mRNA where decoding should begin and where it should end. The selection of the translation start site is usually delineated by the first AUG codon encoding the amino acid methionine. After initiation of translation, the ribosome manufactures the polypeptide by progressing along the mRNA in the 5' to 3' direction, decoding one codon at a time. The final step in the translation process occurs when one of three termination codons occupies the A-site of the ribosome, resulting in hydrolysis of the peptide reviewed in Buckingham et al., 1997). Although translation termination normally occurs after completion of the full-length polypeptide, base substitutions and frameshift mutations in DNA often lead to the synthesis of an mRNA that contains an inappropriate stop codon within its protein coding region. The occurrence of such a premature stop codon arrests translation at the site of early termination and causes the synthesis of a truncated protein and rapid degradation of the mRNA (reviewed in Ruiz-Echevarria et al., 1996; Weng et al., 1997). Interestingly, nonsense and frameshift mutations cause approximately 20-40% of the individual cases of over 240 different inherited diseases (reviewed in McKusick, 1994). Thus, treatment of a number of genetic disorders can be envisioned by promoting nonsense suppression. Nonsense suppression results when a near cognate tRNA successfully competes with the termination factors at a nonsense mutation so that amino acid incorporation into the peptide chain occurs rather than prematurely terminating translation (Fig. 1). Sufficient levels of nonsense suppression allows production of completed polypeptide protein. For many diseases in which only one percent of the functional protein is produced, patients suffer serious disease symptoms, whereas boosting expression to only five percent of normal levels can greatly reduce the severity or eliminate the disease (McKusick, 1994; Cooper etc.). Recent reports have demonstrated that sub-inhibitory concentrations of certain aminoglycosides suppress the translation termination process, resulting in the expression of full-length CFTR and restoring cyclic AMP-activated chloride channel activity (Bedwell et al. 1997; Howard et al., 1996). Thus, identifying and characterizing the factors that regulate the efficiency of the translation termination will be important for

understanding the biology of this process as well as in developing therapeutics for the treatment of a wide array of genetic disorders that arise as a consequence of a nonsense mutations.

Translation termination is carried out by the eucaryotic peptidyl release factors Release Factor 1 (eRF1) and Release Factor 3 (eRF3). Both eRF1 and eRF3 are conserved proteins that interact and promote peptidyl release in eucaryotic cells (Frolova et al. 1994, Stansfield et al. 1995, Zhouravleva et al. 1995). In yeast, eRF1 and eRF3 are encoded by the *SUP45* and *SUP35* genes, respectively (Frolova et al. 1994, Zhouravleva et al. 1995). Sup45p (eRF1) and Sup35p (eRF3) have been shown to interact (Stansfield et al 1995, Paushkin et al 1997a,b). eRF1 contains intrinsic peptide hydrolysis activity while eRF3, which has homology to the translation elongation factor EF1α (Didichenko et al. 1991), demonstrates GTPase activity (Frolova et al. 1996), and enhances the termination activity of eRF1 in a GTP-dependent manner (Zhouravleva et al. 1995).

Factors that modulate the efficiency of translation termination process have been identified (Weng et al., 1996a,b; Czaplinski et al., 1998; Song and Liebman, 1987; All-Robyn et al. 1990). For example, recent results indicate that the Upf1p is a factor that modulates the efficiency of translation termination. Disruption of the *UPF1* gene results in a dramatic stabilization of nonsense-containing mRNAs and promotes suppression of certain nonsense alleles (Leeds et al. 1991, Cui et al. 1995, Czaplinski et al. 1995,1998 Weng et al. 1996a,1996b). Recent results suggest that the Upf1p may modulate the translation termination process by directly interacting with eRF1 and eRF3 (Czaplinski et al., 1998). The Upf1p contains a cysteine- and histidine-rich region near its amino terminus and all the motifs required to be a member of the superfamily group I helicases (Czaplinski et al. 1995,; Weng et al. 1996a,b, 1998, Altamura et al. 1992, Cui et al. 1996, Koonin, 1992, Leeds et al. 1992, Atkin et al. 1995,1997). The yeast Upf1p has been purified and demonstrates RNA-dependent ATPase and helicase activity (Czaplinski et al. 1995, Weng et al. 1996a,b, 1998). A human homologue of the *UPF1* gene, called